Journal of Medical Virology 59:496–501 (1999)

Human Herpesviruses 6 and 7 as Potential Pathogens After Liver Transplant: Prospective Comparison With the Effect of Cytomegalovirus

Paul D. Griffiths,[1]* Mounir Ait-Khaled,[1] Charlotte P. Bearcroft,[2] Duncan A. Clark,[1] Alberto Quaglia,[3] Susan E. Davies,[3] Andrew K. Burroughs,[2] Keith Rolles,[4] I. Michael Kidd,[1] Sophia N. Knight,[1] Shanita M. Noibi,[1] Alethea V. Cope,[1] Andrew N. Phillips,[5] and Vincent C. Emery[1]

[1]*Department of Virology, Royal Free Hospital and Royal Free and University College Medical School, London, England*
[2]*Department of Medicine, Royal Free Hospital and Royal Free and Univeristy College Medical School, London, England*
[3]*Department of Histopathology, Royal Free Hospital and Royal Free and University College Medical School, London, England*
[4]*Department of Academic Surgery, Royal Free Hospital and Royal Free and University College Medical School, London, England*
[5]*Department of Primary Care and Population Sciences, Royal Free Hospital and Royal Free and University College Medical School, London, England*

Because cytomegalovirus (CMV) is an important opportunistic infection after liver transplant, we conducted a prospective study to see if the same applied to human herpesviruses (HHV)-6 and -7. We used polymerase chain reaction (PCR) methods optimised to detect active, not latent, infection and studied patients not receiving antiviral prophylaxis for CMV. Post-transplant, 536 blood samples were tested by PCR (median 7; range 4–50). Active infection with CMV was detected in 28/60 (47%), HHV-6 in 19/60 (32%), and HHV-7 in 29/60 (48%) of patients. The PCR-positive samples were tested by quantitative-competitive PCR to measure the virus load of each betaherpesvirus. The median peak virus load for CMV was significantly greater than that for HHV-6 or HHV-7. Detailed clinicopathological analyses for the whole population showed that CMV and HHV-6 were each significantly associated with biopsy-proven graft rejection. Individual case histories suggested that HHV-6 and HHV-7 may be the cause of some episodes of hepatitis and pyrexia. It is concluded that HHV-6 is a previously unrecognized contributor to the morbidity of liver transplantation, that HHV-7 may also be important and that both viruses should be included in the differential diagnosis of graft dysfunction. ***J. Med. Virol. 59:496–501, 1999.***

KEY WORDS: cytomegalovirus; human herpesvirus-6; human herpesvirus-7

INTRODUCTION

Iatrogenic immunocompromise renders liver transplant patients susceptible to a variety of opportunistic infections. Among opportunistic viruses, cytomegalovirus (CMV) is recognised as the predominant clinical problem, causing fever, hepatitis, pneumonitis, gastrointestinal ulceration, or retinitis (reviewed in Griffiths and Emery [1997]). Because CMV disease is both common and serious, it impacts adversely on the successful outcome of liver transplantation [Falagas et al., 1997; Paya et al., 1993].

CMV (human herpesvirus 5 [HHV-5]) is the prototype member of the betaherpesvirus sub-family of the *Herpesviridae*. Recently, two new human viruses, termed HHV-6 and HHV-7, have been added to the betaherpesviruses based on their genetic relatedness to CMV [Salahuddin et al., 1986; Frenkel et al., 1990]. HHV-6 causes pyrexial illness in childhood, including exanthem subitum and febrile seizures [Yamanishi et al., 1988; Hall et al., 1994]. Reports have also associated HHV-6 with a variety of clinical conditions without proof of an aetiological link [Steeper et al., 1990; Daugherty et al., 1991; Challoner et al., 1995]. In the immunocompromised host, HHV-6 infection has been

Presented in part at the 21st Herpesvirus workshop, 27 July–2 August 1996, DeKalb, Illinois.

Grant sponsor: National Institutes of Health. Grant number: 1RO1 AI33389.

*Correspondence to: Professor Paul D. Griffiths, Department of Virology, Royal Free and University College Medical School, Royal Free Campus, Rowland Hill Street, London, England.

Accepted 12 April 1999

detected in the post-transplant period [Drobyski et al., 1993; Wilborn et al., 1994; Osman et al., 1996] and the virus has been associated with pneumonitis, marrow suppression, and encephalitis after bone marrow transplant [Cone et al., 1993; Drobyski et al., 1993, 1994]. In addition, HHV-6 has been detected in autopsy samples in AIDS patients [Corbellino et al., 1993; Knox and Carrigan, 1994; Clark et al., 1996], and has been proposed as a possible co-factor, accelerating the progress of HIV disease [Griffiths, 1992; Lusso, 1996]. HHV-7 appears to cause some cases of exanthem subitum [Tanka et al., 1994; Torigoe et al., 1995], but otherwise has not been associated with particular diseases.

Because CMV acts as an opportunist after liver transplantation, we conducted a prospective study to determine if HHV-6 and HHV-7 act likewise. Viral DNA of each virus was detected by polymerase chain reaction (PCR) using assays previously shown to detect active but not latent infection [Clark et al., 1996; Kidd et al., 1996; Cope et al., 1997]. In addition, quantitative-competitive-PCR (QCPCR) assays were used to measure the viral load of each virus in blood. We aimed to identify when the DNA of these viruses became detectable post-transplant and whether this change was associated temporally and statistically with particular clinical syndromes. Because some of the morbidity associated with CMV may be caused by secondary phenomena such as fever, graft rejection, or superinfections with bacteria or fungi [Paya et al., 1993; Rubin, 1998], we included these conditions in the clinicopathological analyses. Furthermore, by using multivariate analyses, we aimed to determine if each betaherpesvirus behaved as an independent pathogen or whether disease resulted when two or more viruses interacted.

MATERIALS AND METHODS

Viral Serology

Pre-transplant sera were diluted 1:10 and tested for HHV-6 and HHV-7 antibodies by an indirect immunofluorescence assay (IFA) based on methods described previously [Salahuddin et al., 1986]. The antigen substrates in the IFAs were HHV-6 (AJ strain)-infected J JHAN cells and HHV-7 (MK strain)-infected Sup-T1 cells which were acetone-fixed on 12-well teflon-coated glass slides (Hendley-Essex, UK).

PCR and QCPCR Assays

The PCR assays used to detect the three viruses have been described previously. The primers used in this assays amplify regions of: CMV glycoprotein B gene [Darlington et al., 1991]; U67 gene of HHV-6 [Clark et al., 1996]; and HHV-7 U42 gene [Kidd et al., 1996, 1998]. HHV-6 DNA was classified as variant A or B using a PCR typing method described previously [Kidd et al., 1998]. DNA was extracted from whole blood using silica-matrix affinity columns (Qiagen Ltd, UK) and 30 ng of DNA tested by each of the three PCR assays, an amount of DNA that does not detect latent infections in normal individuals [Clark et al., 1996; Kidd et al., 1996; Cope et al., 1997]. Active betaherpesvirus infection was defined when at least one blood sample was PCR-positive. The CMV [Fox et al., 1992], HHV-6 [Clark et al., 1996], and HHV-7 [Kidd et al., 1996] QCPCR assays have been described previously. The CMV PCR results were performed in real time but the assays for HHV-6 and HHV-7 were carried out retrospectively and so could not influence patient management.

Patients

Patients undergoing liver transplantation at this centre were recruited prospectively into the study, which was approved by the local Ethics Committee. Pre-transplant sera were collected. Samples of whole blood were collected weekly after transplant with the aim of recruiting 60 patients with at least four samples available for PCR analysis.

The clinical management of liver transplant patients involved initial intensive care until the patient was self-ventilating and stable cardiovascularly, with adequate renal function. The immunosuppressive regimen was azathioprine 1.5 mg/kg daily and methylprednisolone 0.8 mg/kg daily, started immediately after transplant. Cyclosporine was started from days 1–3 postoperatively, depending on clinical condition and was given intravenously (IV) (4 mg/kg per day; $n = 47$) or orally (10 mg/kg per day; $n = 13$). Ampicillin 1 g four times a day (qds) IV, netilmicin 3.5 mg/kg IV twice a day (bid) and metronidazole were given for 48 hr as bacterial prophylaxis and amphotericin 5 ml qds for 1 month as fungal prophylaxis. Acyclovir 5 mg/kg was given if the patient was seropositive for herpes simplex virus (HSV) preoperatively and was continued orally, 200 mg qid for 1 month. No prophylaxis or preemptive therapy was given for CMV. Liver biopsies were protocolled for days 5, 10, 15, and 25 and were also taken as required for the clinical management of the patient. If moderate or severe rejection was diagnosed histologically or suspected clinically, methylprednisolone 1 g daily was prescribed for 3 days and repeated if rejection persisted. Second-line treatment for rejection was IV OKT3 antibody for 5 days. Cyclosporin was changed to FK506 0.1 mg/kg per day if rejection persisted.

Clinical Features

For each patient, we recorded prospectively: peak maximum daily oral temperature taken as an inpatient; daily biochemical tests of liver function; rejection episodes (their number and histological findings); and doses of augmented immunosuppressive drugs received. CMV disease was diagnosed according to criteria defined at the CMV International Workshop, requiring that CMV be detected in tissues (biopsies, bronchoalveolar lavage) taken from the affected organ [Ljungman and Plotkin, 1995].

Definition of a Peak in Alanine Aminotransferase (ALT) Values

Of the multiple liver function tests, values of daily ALT were chosen as a marker of hepatocellular damage

and plotted against time. A peak was defined as a transient elevation in which the mean of the maximum value and the subsequent value was both at least 1.5 times higher than the previous lowest level and greater than 40 units/L.

A "virus-associated ALT peak" was defined as one occurring within 7 days of PCR positivity for any virus.

Histological Grading of Liver Biopsies

All liver biopsies from these patients were reviewed by two observers (AQ and SED), who were aware of the patient's clinical course but unaware of the PCR results for HHV-6 or HHV-7.

Statistical Methods

Categorical variables were compared using chi-squared or Fisher's exact test. Two-group comparisons of continuous variables were performed using the Mann–Whitney *U* test. Survival analyses (not all of which are shown) were performed using standard techniques, including Kaplan–Meier estimation, log-rank tests and Cox proportional hazards models. Associations between presence of betaherpesviruses and continuous measures of morbidity (number of peaks in ALT; numbers of days of fever, etc.) were assessed using simple and multivariate linear regression after log transformation of the continuous outcome variable.

RESULTS

Patient Recruitment

From 1 August 1993 patients were followed prospectively. The 60th eligible patient was transplanted on 11 October 1995. Between these dates, 135 liver transplants were performed in 120 patients, with 15 patients having two transplants. We studied 60 patients having 60 transplants, 8 of whom had had a previous liver transplant. The remaining patients were excluded because fewer than 4 samples had been received. The demographic characteristics of the 60 study patients are shown in Table I.

TABLE I. Baseline Demographic Characteristics of Patients in the Study

Characteristic	$N = 60$
Mean age (years) (SEM)	44.1 (1.7)
Male sex	41%
Race	
White	46
Hispanic white	0
Black	2
Chinese	0
Indian sub-continent	12
Diagnosis of underlying liver disease	
Primary biliary cirrhosis	7
Primary sclerosing cholangitis	10
with cholangiocarcinoma	1
Hepatitis C	16
with chronic alcoholic liver disease	3
with hepatitis B and hepatitis D	1
Hepatitis B	2
Alcoholic liver disease	9
with hepatocellular carcinoma	1
Cryptogenic cirrhosis	2
Fulminant liver failure	4
Glycogen storage disease	2
Wilson's disease	1
Chronic active hepatitis	1

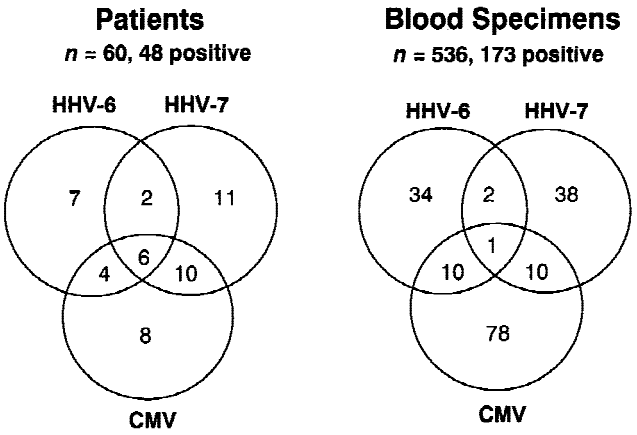

Fig. 1. Venn diagrams illustrating the number of patients ($n = 60$) or number of blood samples ($n = 536$) that were polymerase chain reaction (PCR)-positive for any combination of betaherpesviruses.

Pre-Transplant Serology

Of the 60 patients, 42 (70%) were CMV seropositive, 55 (92%) were HHV-6 seropositive, and 55 (92%) were HHV-7 seropositive. Sera were available from 26 donors; 16/26 (62%), 24/26 (92%), and 26/26 (100%) were seropositive for CMV, HHV-6, and HHV-7, respectively.

Qualititative PCR Testing

A total of 536 blood samples were tested from the 60 patients for the three betaherpesviruses (median number of samples per patient = 7; range 4–50). CMV DNA was detected in 28/60 (47%) of patients, HHV-6 DNA in 19/60 (32%) of patients, and HHV-7 DNA in 29/60 (48%). DNA from one or more virus was detected in 48/60 (80%) patients and 173/536 (32%) samples (Fig. 1). Twenty-two of 60 (37%) patients but only 23/536 (4%) of samples were PCR positive for more than one virus, indicating that most patients infected actively with more than one betaherpesvirus were infected sequentially and not concurrently. Among those who were PCR positive for each virus, the median time to first PCR positivity was 36 days (range 1–338) for CMV, 20 days (range 1–125) for HHV-6, and 26 days (range 1–227) for HHV-7. In 3 patients, HHV-6 PCR was positive immediately after transplant and remained positive on all occasions (total number of bloods tested 7, 7, and 6, respectively). HHV-6 DNA from 8 patients was shown to be variant B in all cases.

Quantitative PCR Testing

The maximum virus loads found in each patient are shown in Figure 2A. The median quantity of CMV (5.18

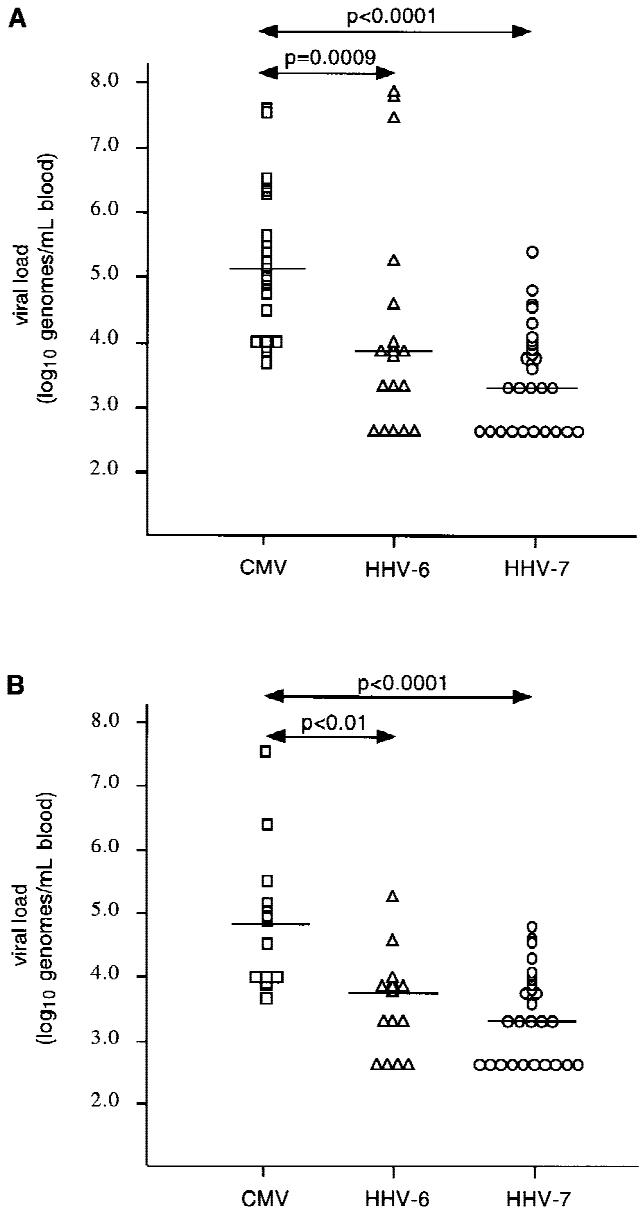

Fig. 2. The maximum viral load of cytomegalovirus (CMV), human herpesvirus (HHV)-6 or -7 found in patients after liver transplantation. The horizontal bars indicate the median value for each betaherpesvirus. **A:** Data from all patients. **B:** Data excluding patients with primary CMV, HHV-6, or HHV-7 infection. (B) also excludes three patients, discussed in the text, with persistent high level HHV-6 DNA.

$\log_{10}$ genomes/ml) was significantly greater than that of either HHV-6 ($P = .0009$) or HHV-7 ($P < .0001$). The median virus loads of HHV 6 (3.81 $\log_{10}$ genomes/ml) and HHV-7 (3.30 $\log_{10}$ genomes/ml) were not significantly different from each other. The three patients with persistent HHV-6 PCR positivity had the highest viral loads shown in Figure 2. When these three patients and those with primary CMV ($n = 12$), primary HHV-6 ($n = 1$), or primary HHV-7 ($n = 1$) infections were excluded, the differences in peak viral loads remained significantly different (Fig. 2B).

TABLE II. Associations Between Betaherpesviruses and Relative Episodes of Biopsy-Proven Graft Rejection

Virus examined in model	Univariate			Multivariate		
	RER	*P*	95% CI	RER	*P*	95% CI
CMV	1.90	.009	1.25–2.54	2.13	.02	1.25–3.01
HHV-6	1.81	.01	1.19–2.42	2.18	.02	1.25–3.11
HHV-7	1.12	.64	0.61–1.63	1.13	.77	0.25–2.01

RER, relative episodes of rejection; CI, confidence interval; CMV, cytomegalovirus; HHV, human herpesvirus.

Clinicopathological Correlations

We reviewed the patients' records that were available from 57/60 patients. Altered liver biochemistry, pyrexia, or gastrointestinal system abnormalities dominated the clinical picture and each virus was temporally associated with some of these episodes. The pyrexial episodes associated with HHV 6 were not explained by bacteremias (data not shown). To analyze changes in liver function, the major clinical feature, serum ALT values were plotted against time post-transplant and defined a total of 101 ALT peaks (median 1; range 0–9) in the 60 patients. Liver biopsies had been taken during 67 of the 101 ALT peaks. Rejection was graded as mild in 25 biopsies, moderate in 8, severe in 1, and chronic in 1, whereas the remaining 32 biopseis showed no rejection. We examined whether rejection was more or less likely to be found in groups of patients who were PCR positive for each betaherpesvirus at any time post-transplant. As shown in Table II, CMV and HHV-6 were associated significantly with biopsy-proven graft rejection; effects shown to be independent in multivariate analyses. No significant association was seen for HHV-7.

The temporal relationships were examined between detection of betaherpesviruses, liver biopsy results, and timing post-transplant of "virus-associated ALT peaks." A total of 22 peaks (14 patients) were CMV associated, 9 peaks (8 patients) HHV-6 associated and 12 peaks (10 patients) HHV-7 associated. Two peaks in one patient were each associated with both CMV and HHV-6, whereas two peaks in a second patient were each associated with both CMV and HHV-7. Thus, a total of 39 ALT peaks (in 24 patients) were associated with betaherpesviruses. Liver biopsies were taken during 27/38 (71%) virus-associated peaks and 40/63 (63%) of the remaining ALT peaks. The CMV-associated peaks occurred significantly later (median 46 days; range 9–217) than those associated with HHV-6 (median 20 days; range 7–79; $P = .003$) or HHV-7 (median 16 days; range 3–169; $P = .023$). Of the 6 HHV-6-associated peaks where biopsies were taken, all showed rejection (3 graded mild, 3 moderate). In contrast, of the 14 CMV-associated peaks, 7 (50%) showed rejection (4 graded mild, 2 moderate, 1 chronic), whereas of the 9 HHV-7-associated peaks, 4 (44%) showed rejection (all graded mild). Because CMV is a known cause of hepatitis and was confirmed by histological identifica-

tion of CMV inclusion bodies in 4 patients, it is possible that the HHV-7-associated peaks for which no rejection was observed represent HHV-7-associated hepatitis. There was no evidence that the 3 patients with persistent HHV-6 affected these analyses. Specifically, 1 of these patients had no ALT peaks post-transplant, the second had a peak at day 22 with mild rejection shown on biopsy, and the third had an ALT peak on day 17 with no biopsy taken.

Similar analyses were carried out for other endpoints, including doses of immunosuppressants received, days of fever, etc., but none showed significant associations with HHV-6 or HHV-7. Likewise, exclusion of patients seropositive for hepatitis C pretransplant did not change any of these results.

Eleven cases of CMV disease occurred (4 pneumonitis, 6 hepatitis, 1 gastric). In multivariate statistical models, there was no association between the presence of HHV-6 and/or HHV-7 together with CMV DNAemia in patients who had CMV disease. A total of 15 deaths occurred and were evenly distributed among the different virus groups, ranging from 4/26 (15%) in patients with one betaherpesvirus to 3/6 (50%) in patients with all three betaherpesviruses.

DISCUSSION

The results described above show that few patients remain free of betaherpesviruses after liver transplantation. All three viruses behaved as classical, opportunistic herpesviruses in that virus DNA was undetectable at the time of transplant (a finding that confirms that the PCR methods used do not detect latent virus), appeared once the patients were immunocompromised, and then disappeared when immune function improved. The only exception to this observation was in three patients with persistent PCR positivity for HHV-6 associated with high viral load, which has also been observed in the peripheral blood of a normal individual and patients with lymphomas [Torelli et al., 1995; Clark et al., 1996]. The QCPCR results showed that the median virus load for CMV was significantly greater than that for HHV-6 or HHV-7. The results remained significant when patients with primary CMV infections were excluded, implying that the replicative ability of betaherpesviruses in liver transplant patients can be ranked: CMV > HHV-6 >HHV-7.

Clinicopathological analyses identified graft dysfunction as the major clinical presentation associated with betaherpesviruses. The pathogenesis of liver dysfunction appears to be distinct for each betaherpesvirus, because CMV and HHV-6 were associated with graft rejection whereas no such association was seen for HHV-7. In contrast, both CMV and HHV-7 were associated statistically with abnormal liver function tests in the absence of rejection. Because CMV is a known cause of hepatitis, it will be interesting to investigate whether HHV-7 DNA is found in liver biopsies of these patients, implicating it as an unrecognised agent in unexplained hepatitis. Nevertheless, the episodes of liver dysfunction appeared to be similar clinically, as shown by the finding that graft biopsies were deemed to be indicated in 27/38 (71%) virus-associated ALT peaks compared with 40/63 (63%) non-virus-associated ALT peaks. We suggest that HHV-6 may act to precipitate or augment graft rejection and note a previous association between HHV-6 and graft-versus-host disease following bone marrow transplantation [Appleton et al., 1995]. Although additional natural history studies are required to test these suggestions in liver transplant patients and extend the work to other groups of allograft recipients, we suggest that HHV-6 and HHV-7 should be included in the differential diagnosis of impaired liver function post-transplant. One way of addressing a causal relationship between HHV-6 and graft rejection would be to conduct placebo-controlled prophylaxis trials of antiviral drugs with activity against this virus in liver transplant patients to determine if this putative pathological effect could be reduced. Some licensed antiherpes drugs have activity against both CMV and HHV-6 in vitro and so are possible candidates for such controlled trials [Burns and Sandford, 1990; Agut et al., 1991; Dockrell et al., 1997]. We suggest that future prophylaxis trials designed to inhibit CMV replication should evaluate whether any clinical benefit seen can be attributed to inhibition of HHV-6 or HHV-7.

This study did not provide evidence for a major pathogenic effect of HHV-7 infection in this population of patients. However, it would be premature to conclude that HHV-7 has no pathological potential, especially given its relative frequency, the fact that exanthem subitum has been attributed to this virus [Tanaka et al., 1994; Torigoe et al., 1995], its association here with individual episodes of hepatitis, and that relatively few cases of primary infection have been identified in the immunocompromised host. Furthermore, morbidity may be increased among patients infected with more than one virus, so it remains possible that HHV-7 might interact with CMV and/or HHV-6 to increase morbidity. One recent study [Dockrell et al., 1997] in liver transplant patients reported that CMV disease was more frequent in those with serological evidence of HHV-6 infection. Analysis of the relationship between CMV disease and presence of HHV-6 or HHV-7 DNAemia in the patients described here did not reveal any significant association, that is, CMV DNAemia was the single most important risk factor for CMV disease. Nevertheless, among the CMV D+R+ group, an increased incidence of CMV disease was seen in patients who were PCR positive for HHV-6 or HHV-7 post-transplant compared with those who were only CMV PCR positive (1/5 vs 7/12; P = NS). Clearly, larger numbers of patients in such subgroups are required but further investigations in distinct patient groups using PCR-based detection of viruses post-transplant have the potential to better define in vivo possible temporal interactions between members of the *Betaherpesvirinae*.

REFERENCES

Agut H, Aubin JT, Huraux JM. 1991. Homogeneous susceptibility of distinct human herpesvirus 6 strains to antivirals in vitro. J Infect Dis 163:1382–1383.

Appleton AL, Sviland L, Peiris JS, Taylor CE, Wilkes J, Green MA, Pearson ADJ, Kelly PJ, Malcolm AJ, Proctor SJ, Hamilton PJ, Cant AJ. 1995. Human herpes virus-6 infection in marrow graft recipients: role in pathogenesis of graft-versus-host disease. Newcastle upon Tyne Bone Marrow Transport Group. Bone Marrow Transplant 16:777–782.

Burns WH, Stanford GR. 1990. Susceptibility of human herpesvirus 6 to antivirals in vitro. J Infect Dis 162:634–637.

Challoner PB, Smith KT, Parker JD, MacLeod DL, Coulter SN, Rose TM, Schultz ER, Bennett JL, Garber RL, Chang M, Schad PA, Stewart PM, Nowkinski RC, Brown JP, Burmer GC. 1995. Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. Proc Natl Acad Sci USA 92:7440–7444.

Clark DA, Ait-Khaled M, Wheeler AC, Kidd IM, McLaughlin JE, Johnson, Griffiths PD, Emery VC. 1996. Quantification of human herpesvirus 6 in immunocompetent persons and post-mortem tissues from AIDS patients by PCR. J Gen Virol 77:2271–2275.

Cone RW, Hackman RC, Huang ML, Bowden RA, Meyers JD, Metcalf M, Zeh J, Ashley R, Corey L. 1993. Human herpesvirus 6 in lung tissue from patients with pneumonitis after bone marrow transplantation. N Engl J Med 329:156–161.

Cope AV, Sabin C, Burroughs A, Rolles K, Griffiths PD, Emery VC. 1997. Inter-relationships between quantity of human cytomegalovirus DNA in blood, donor/recipient serostatus and administration of methylprednisolone as risk factors for HCMV disease following liver transplantation. J Infect Dis 176:1484–1490.

Corbellino M, Lusso P, Gallo RC, Parravicini C, Galli M, Moroni M. 1993. Disseminated human herpesvirus 6 infection in AIDS. Lancet 342:1242.

Darlington J, Super M, Patel K, Grundy JE, Griffiths PD, Emery VC. 1991. Use of the polymerase chain reaction to analyse sequence variation within a major neutralizing epitope of glycoprotein B (gp58) in clinical isolates of human cytomegalovirus. J Gen Virol 72:1985–1989.

Daugherty SA, Henry BE, Peterson DL, Swarts RL, Bastien S, Thomas RS. 1991. Chronic fatigue syndrome in northern Nevada. Rev Infect Dis 13(Suppl 1):S39–S44.

Dockrell DH, Prada J, Jones MF, Patel R, Badley AD, Harmsen WS, Ilstrup DM, Wiesner RH, Krom RAF, Smith TF, Paya CV. 1997. Seroconversion to human herpesvirus 6 following liver transplantation is a marker of cytomegalovirus disease. J Infect Dis 176: 1135–1140.

Drobyski WR, Dunne WM, Burd EM, Knox KK, Ash RC, Horowitz MM, Flomenberg N, Carrigan DR. 1993. Human herpesvirus-6 (HHV-6) infection in allogeneic bone marrow transplant recipients: evidence of a marrow-suppressive role for HHV-6 in vivo. J Infect Dis 167:735–739.

Drobyski WR, Knox KK, Majewski D, Carrigan DR. 1994. Brief report: fatal encephalitis due to variant B human herpesvirus-6 infection in a bone marrow-transplant recipient. N Engl J Med 330: 1356–1360.

Falagas ME, Snydman DR, Griffith J, Ruthazer R, Werner BG, and the Boston Center for Liver Transplantation CMVIG Study Group. 1997. Effect of cytomegalovirus infection status on first-year mortality rates among orthotopic liver transplant recipients. Ann Intern Med 126:275–279.

Fox JC, Griffiths PD, Emery VC. 1992. Quantification of human cytomegalovirus DNA using the polymerase chain reaction. J Gen Virol 73:2405–2408.

Frenkel N, Schirmer EC, Wyatt LS, Katsafanas G, Roffman E, Danovich RM, June CH. 1990. Isolation of a new herpesvirus from human CD4+ T cells. Proc Natl Acad Sci USA 87:748–752.

Griffiths PD. 1992. Studies to define viral cofactors for human immunodeficiency virus. Infect Agents Dis 1:237–244.

Griffiths PD, Emery VC. 1997. Cytomegalovirus. In: Richman DD, Whitley RJ, Hayden FG, editors. Clinical virology. New York: Churchill Livingstone. p 445–470.

Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, Knott A, Dewhurst S, Insel RA, Epstein LG. 1994. Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. N Engl J Med 331:432–438.

Kidd IM, Clark DA, Ait-Khaled M, Griffiths PD, Emery VC. 1996. Measurement of human herpesvirus 7 load in peripheral blood and saliva of healthy subjects by quantitative polymerase chain reaction. J Infect Dis 174:396–401.

Kidd IM, Clark DA, Bremner JAG, Pillay D, Griffiths PD, Emery VC. 1998. A multiplex PCR assay for the simultaneous detection of human herpesviruses 6 & 7, with typing of HHV 6 by enzyme cleavage of PCR products. J Virol Methods 70:29–36.

Knox KK, Carrigan DR. 1994. Disseminated active HHV-6 infections in patients with AIDS. Lancet 343:577–578.

Ljungman P, Plotkin SA. 1995. Workshop of CMV disease: definitions, clinical severity scores and new syndromes. Scand J Infect Dis s99:87–89.

Lusso P. 1996. Human herpesvirus 6 (HHV 6). Antivir Res 31:1–21.

Osman HK, Peiris JS, Taylor CE, Warwicker P, Jarrett RF, Madeley CR. 1996. "Cytomegalovirus disease" in renal allograft recipients: is human herpesvirus 7 a co-factor for disease progression? J Med Virol 48:295–301.

Paya CV, Wiesner RH, Hermans PE, Larson-Keller JJ, Ilstrup DM, Krom RA, Rettke S, Smith TF. 1993. Risk factors for cytomegalovirus and severe bacterial infections following liver transplantation: a prospective multivariate time-dependent analysis. J Hepatol 18:185–195.

Rubin RH. 1998. Cytomegalovirus disease and allograft loss after organ transplantation. Clin Infect Dis 26:871–873.

Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B, Gallo RC. 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science 234:596–601.

Steeper TA, Horwitz CA, Ablashi DV, Salahuddin SZ, Saxinger C, Saltzman R, Schwartz B. 1990. The spectrum of clinical and laboratory findings resulting from human herpesvirus-6 (HHV-6) in patients with mononucleosis-like illnesses not resulting from Epstein-Barr virus or cytomegalovirus. Am J Clin Pathol 93:776–783.

Tanaka K, Kondo T, Torigoe S, Okada S, Mukai T, Yamanishi K. 1994. Human herpesvirus 7: another causal agent for roseola (exanthem subitum). J Pediatr 125:1–5.

Torelli G, Barozzi P, Marasca R, Cocconcelli P, Merelli E, Ceccherini-Nelli L, Ferrari S, Luppi M. 1995. Targeted integration of human herpesvirus 6 in the p arm of chromosome 17 of human peripheral blood mononuclear cells in vivo. J Med Virol 46:178–188.

Torigoe S, Kumamoto T, Koide W, Taya K, Yamanishi K. 1995. Clinical manifestations associated with human herpesvirus 7 infection. Arch Dis Child 72:518–519.

Wilborn F, Brinkmann V, Schmidt CA, Neipel F, Gelderblom H, Siegert W. 1994. Herpesvirus type 6 in patients undergoing bone marrow transplantation: serologic features and detection by polymerase chain reaction. Blood 83:3052–3058.

Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, Kurata T. 1988. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet 1:1065–1067.